Cyanotoxins: A Poison that Frees Phosphate

Autotrophic organisms obtain phosphorus from the environment by secreting alkaline phosphatases that act on esters, resulting in inorganic phosphate that is then taken up. New work shows that the cyanobacterium *Aphanizomenon ovalisporum* obtains inorganic phosphate by secreting the cyanotoxin cylindrospermopsin, which induces alkaline phosphatase in other phytoplankton species.

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Phytoplankton living within the first 100 metres of the water column, in the ocean and in continental water bodies, account for about half of global primary productivity. The cyanobacteria and algae that comprise the phytoplankton can broadly be considered as photolithotrophs, i.e. growing with light as the energy source and inorganic compounds as the source of nutrient elements. This has long been known to be an over-simplification, and recent work has shown an increasingly complex picture of nutrition of phytoplankton in the natural environment. One aspect of this complexity is the acquisition of phosphorus from external phosphate esters by secretion of an alkaline phosphatase, which occurs when the cells are phosphorus limited. The resulting inorganic phosphate is taken up by the cells producing the phosphatase, or even by an alga that did not produce the extracellular phosphatase. In a recent issue of *Current Biology*, Bar-Yosef *et al.* [1] report an interesting twist on this pathway by showing the expression of the phosphatase in response to a signal in the form of a cyanotoxin from a cyanobacterium. The cyanobacterium in question is *Aphanizomenon ovalisporum* (Figure 1), and while this organism can produce extracellular phosphatase, its first recourse is to cause cells of some other phytoplankton species to make extracellular phosphatase whose inorganic phosphate product can, in part, be used by *A. ovalisporum*, as well as other organisms.

The production by phytoplankton of organic compounds that damage some sympatric species is well documented. Some compounds with a complex and specific synthetic pathway have no well authenticated biological effects other than influencing other organisms: examples are the toxins produced by many cyanobacteria (e.g., microcystin), dinoflagellates and a few diatoms (domoic acid). The work of Bar-Yosef *et al.* [1] identified the cyanotoxin cylindrospermopsin as having an influence on other phytoplankton. More specifically, cylindrospermopsin induces the production of alkaline phosphatase even when the responding algae are not phosphorus-deficient, which can only be regarded as toxicity if resource diversion to produce alkaline phosphatase decreases the fitness of the alga. There is evidence that cyanobacterial products other than cylindrospermopsin are involved in the signalling and that natural phytoplankton organisms are less responsive to cylindrospermopsin than is a green algae that has been in culture for some time, suggesting the loss of partial resistance.

The production of extracellular enzymes is widespread in eukaryotic algae and cyanobacteria. The four elements that most frequently limit phytoplankton growth in nature are carbon, nitrogen, phosphorus and iron, and in all four cases there are mechanisms of acquisition of the element that involve enzyme-mediated chemistry outside the cell (or with at least the active site of the enzyme facing the environment). In all cases there is the possibility of loss to the bulk medium of the chemical species produced by the enzyme as well as of uptake by the organism producing the enzyme.

For inorganic carbon, many algae take up through their carbon dioxide concentrating mechanism both carbon dioxide and bicarbonate [2]; these two species are often taken up at approximately equal rates from a seawater or alkaline freshwater medium despite the much greater concentration of bicarbonate than of carbon dioxide in an equilibrium solution. The slow, uncatalysed equilibration of carbon dioxide and bicarbonate, and their low aqueous diffusion constants, would mean carbon dioxide depletion in the neighbourhood of a photosynthesising cell, a situation avoided by the presence of an extracellular carbonic anhydrase enzyme [2]. Some of the carbon dioxide generated in this way is presumably lost from the cell surface [2], just as happens with carbon dioxide generated by intracellular carbonic anhydrase in some algal cells that take up only bicarbonate and lack external carbonic anhydrase [3].

In the case of nitrogen, in addition to the capacity to take up combined inorganic nitrogen (i.e., ammonium and, very generally, nitrate and nitrite) many algae can take up and assimilate one or more of urea, amino acids and ureides. However, some phytoplankton cells have an extracellular L-amino-acid oxidase which yields an oxo-acid (not taken up) and ammonium, which can be taken up by the cells producing the enzyme but which can also be lost to the medium and could be acquired by some other organism [4].

For phosphate, most of the organic phosphate in natural waters exists as phosphate esters, and the esters are generally thought not to be taken up directly but to be hydrolysed by an extracellular alkaline phosphatase

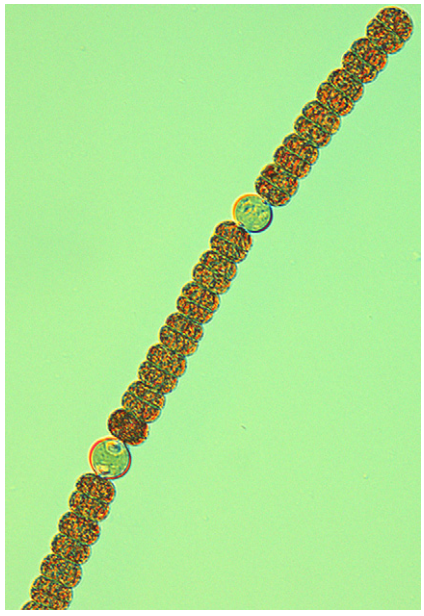

Figure 1. *Aphanizomenon ovalisporum*. (photo courtesy of A. Kaplan)

whose production is increased by phosphorus limitation in many phytoplankton and bacteria. The alkaline phosphatase is secreted; some remains attached to the cell, some is released into the medium [5–9]. The phosphatase yields an organic moiety, which is not generally taken up, and inorganic phosphate, which can be transported into the cell, although some is likely to be lost to the bulk medium.

Finally, iron in the surface ocean is predominantly in the oxidised ferric form bound to organic ligands. The predominant mechanisms of iron acquisition by phytoplankton appears to be the enzymic reduction of ferric iron at the cell surface, followed by uptake of ferrous iron [10], and this process can have an efficiency (percentage of ferric iron reduced that enters the cells as ferrous iron) of up to 30% [11]. Coastal cyanobacteria obtain this iron by secreting siderophores, which are iron-chelating compounds with a higher affinity for ferric iron than the ligands in the water body. Siderophore–iron complexes can then be taken up by the cyanobacteria and also used by some eukaryotes [10]. This mechanism involves at least 50 mole siderophore secreted per mole iron taken up by the cell, with a cost in terms of nitrogen in the siderophore of at least 3% of that assimilated during cell growth for the conditions assumed in the analysis [11]. This mechanism, involving secretion of a relatively low molecular mass organic molecule, has similarities to the secretion of toxins such as cylindrospermopsin and domoic acid.

This background discussion shows that the production of extracellular enzymes that facilitate the acquisition of nutrient elements is widespread in phytoplankton, although the extent of production of the exo-enzymes varies among taxa. This variation opens the way to cheating, i.e. acquisition of the usable product by organisms that produce little or no extracellular enzyme activity [12–14]. The occurrence of this cheating is clearly illustrated by the work of Bar-Yosef *et al.* [1], in which the cyanobacterium causes other organisms to over-produce alkaline phosphatase. Such a strategy is only evolutionarily viable if the resource cost per unit phosphorus acquired to the cyanobacterium of making the cyanotoxin is less than that of making its own phosphatase. Bar-Yosef *et al.* [1] have gone part of the way to showing this decreased resource cost by calculating that the cost to the cyanobacterium in units of nitrogen of making the cylindrospermopsin found in the water body is less than half the nitrogen cost of making the alkaline phosphatase. Similar considerations apply to the energy cost, especially since the non-ribosomal synthesis of the toxin uses less energy per amino-acyl group added than does the ribosomal synthesis of acid phosphatase.

These considerations show that there are indeed apparent economies in resource costs of the cynaobacterium using a secreted semiochemical (cylindrospermopsin) to cause another organism to make alkaline phosphatase rather than the cyanobacterium making its own enzyme. However, the comparison of the resource costs was not on the basis of unit phosphorus acquisition by the cyanobacterium, and it is worth considering the extent to which the apparent economies could be offset by not all of the cylindrospermopsin molecules inducing alkaline phosphatase, and by less inorganic phosphate being made available to the cyanobacterium by alkaline phosphatase of other organisms rather than by alkaline phosphatase on the cyanobacterium. In other words, how significant is dilution of both the signal molecule cylindrospermopsin and the usable resource (inorganic phosphate) for operation of the mechanism? Another apparently unresolved question is the relationship between *Aphanizomenon* density (cells per unit volume), steady-state cylindrospermopsin (and other relevant cyanobacterial products) concentration and induction of alkaline phosphatase in eukaryotic phytoplankton. Data on this would help to set limits on the stoichiometry of cyanotoxin production and alkaline phosphatase synthesis, using what data are available on the lifetime of the cyanotoxin and the phosphatase. Yet another question is the effectiveness of a given alkaline phosphatase activity secreted by a eukaryotic phytoplankton organism in supplying inorganic phosphate to *Aphanizomenon* relative to the effectiveness of the same phosphatase activity secreted by the cyanobacterium, and the extent to which the alkaline phosphatase from the two sources is released from the cell surface and occurs free in the medium [8]. The answers to these last two questions would partly address the dilution considerations mentioned above.

More widely, do low molecular mass organic compounds secreted by cyanobacteria and algae have any other roles in nutrition by influencing other organisms? It has been known since phytoplankton cells began to be cultured in defined media that a significant fraction of the strains investigated required external supply of (are said to be auxotrophic for) one or more of the B vitamins that are needed for metazoans, including man, i.e. vitamin B12, biotin and thiamine. In the natural phytoplankton environment the vitamins are supplied by bacteria, and it has been contended that the phytoplankton cells requiring the vitamins have symbiotic relations with bacteria producing one or more vitamins in exchange for organic carbon from the alga [15]. However, it has been plausibly argued that the concentration of vitamin B12 in oceanic and coastal waters is adequate to supply this vitamin to these algae that cannot synthesise it without the need for symbiosis [16]. Since the auxotrophic algae depend on leaking, or secretion, of vitamins from the producer organism into the medium, might this secretion be stimulated by substances secreted by auxotrophic algae, as is the case for ‘enslavement’ in phosphorus supply?

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Motor Control: Exploring the Neurochemistry of Subliminal Inhibition

A new study links individual differences in unconsciously triggered motor control to variability in GABA neurotransmitter concentration in the supplementary motor area of the human brain.

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It has been known for some time that masked 'prime' stimuli, presented below the threshold of conscious perception, can bias behavioral responses to subsequent probe stimuli, facilitating prime-compatible and hindering prime-incompatible responses, presumably by partially activating prime-compatible motor pathways [1]. More recently, this picture has been qualified by the intriguing observation that, with certain prime-to-probe delays, this classic effect can be reversed, resulting in a 'negative compatibility effect', where it is the prime-*compatible* probe response that is slowed [2]. This effect has been attributed to an unconscious act of motor control, consisting of the automatic inhibition of a partially activated response if it is no longer supported by unequivocal perceptual input [2,3].

The negative compatibility effect has attracted much attention [4,5], for several reasons. First, the very notion of unconscious or automatic 'control' sets the pulses of cognitive psychologists racing, because it represents an oxymoron *vis-à-vis* the traditional view of control processes being, by definition, volitional and effortful [6,7]. In fact, in combination with other recent work on seemingly 'automatic' strategic control [8–11], research employing the negative compatibility effect has contributed forcefully to the ongoing erosion of the traditional dichotomy between 'automatic' and 'controlled' processing [12,13]. Second, if the negative compatibility effect were an unconscious automatic mechanism, and was thus presumably immune to the noisy caprice of volitional processes, it could potentially serve as an attractive measure of individual differences in inhibitory control in the clinical domain [14,15]. On both counts, a thorough understanding of the neural mechanisms underlying the negative compatibility effect would be of great interest.

In this issue of *Current Biology*, Boy and colleagues [16] make an enlightening contribution to this quest, by harnessing an innovative combination of behavioral and neuroimaging techniques. Previous lesion data had implicated the supplementary motor area (SMA) as a key region in producing the negative compatibility effect [17]. Armed with these data, the authors set out to ask what, in this field, is a highly important, but rarely posed, question: can individual differences in behavior be explained by regionally specific variability in neurochemistry? And specifically, might individual differences in the expression of subliminal motor control be related to variability in the concentration of the brain's primary inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), in the SMA? Boy *et al.* [16] pursued this question by combining careful behavioral experimentation with magnetic resonance spectroscopy (MRS), an imaging technique that exploits the fact that different metabolites in the brain have different resonant frequencies, thus producing MR spectra with peaks that reflect the relative concentration of different molecules, including the neurotransmitters glutamate and GABA [18].

Boy *et al.* [16] first established that the negative compatibility effect represents a stable, trait-like measure, displaying high test-retest reliability within individuals. Subsequently, they employed structural magnetic resonance imaging (MRI) for anatomically localizing the SMA in each participant, in order to then acquire MRS data from a cortical volume centered on this area. Finally, the quantification of the area under the GABA peak in each individual's MR spectrum enabled Boy and colleagues to assess the relationship between individual differences in SMA GABA concentration and subliminal motor inhibition, as gauged by the negative compatibility effect. The results indicated a strong inverse relationship, which proved to be robust across two independent subject cohorts. Importantly, this correlation between GABA and automatic motor control was regionally specific: MRS data collected from a number of control regions that are associated with various forms of action control, including the anterior cingulate, dorsolateral prefrontal cortex, parietal cortex, and inferior frontal gyrus, all yielded null results. Similarly, the association between SMA GABA